Synthesis of *N*-[1-(2-Thienyl)cyclohexyl]piperidine (TCP) Based Irreversible Alkylators of the Phenylcyclidine (PCP) Recognition Site

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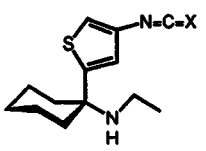
Abstract: Four new thienylcyclohexylamines {*N*-[1-(4-isothiocyanato/isoselenocyanato-2-thienyl)cyclohexyl]piperidine and 2-[1-(ethylaminocyclohexyl)-4-isothiocyanato/isoselenocyanatothiophene} have been synthesized and examined for their ability to alkylate the MK-801/PCP recognition sites associated with the *N*-methyl-D-aspartate receptor-ion channel complex.

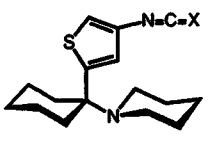
The N-methyl-D-aspartate (NMDA) receptor-ion channel complex represents one subtype of glutamate receptor found in the brain and plays a fundamental role in processes related to memory and learning[1]. As a consequence of these and other physiological properties, considerable interest exists in the isolation and detailed molecular characterization of this multi-subunit protein complex. To separate this particular complex from the multitude of other proteins it is necessary to attach to it a radiolabel which will permit its detection during the purification operations. This is generally achieved through the use of radiolabeled analogues of compounds known to bind selectively and irreversibly to the receptor complex. To date a variety of chemical ligands capable of irreversible labeling of the NMDA receptor have been examined. These ligands include a photoactivatable meta-azido derivative of phencyclidine (PCP)[2], the corresponding meta-isothiocyanato derivative ("metaphit")[3], and an azido derivative of the Merck drug MK-801[4]. It should be noted that the PCP recognition site associated with the NMDA complex is located somewhere within its Na^+/Ca^{2+}-permeable ion channel[5].

In this paper we present the chemistry developed for the synthesis of the 4-isothiocyanato as well as isoselenocyanato derivatives of N-[1-(2-thienyl)cyclohexyl]piperidine (TCP) and thienylcyclohexylethylamine (TCE). *One of these compounds has proven to be the most efficient alkylating agent reported to date for binding to the PCP/MK-801 recognition sites.*

Potential Irreversible Alkylators of the NMDA Receptor-Associated PCP Recognition Sites.



1a, X = S
1b, X = Se



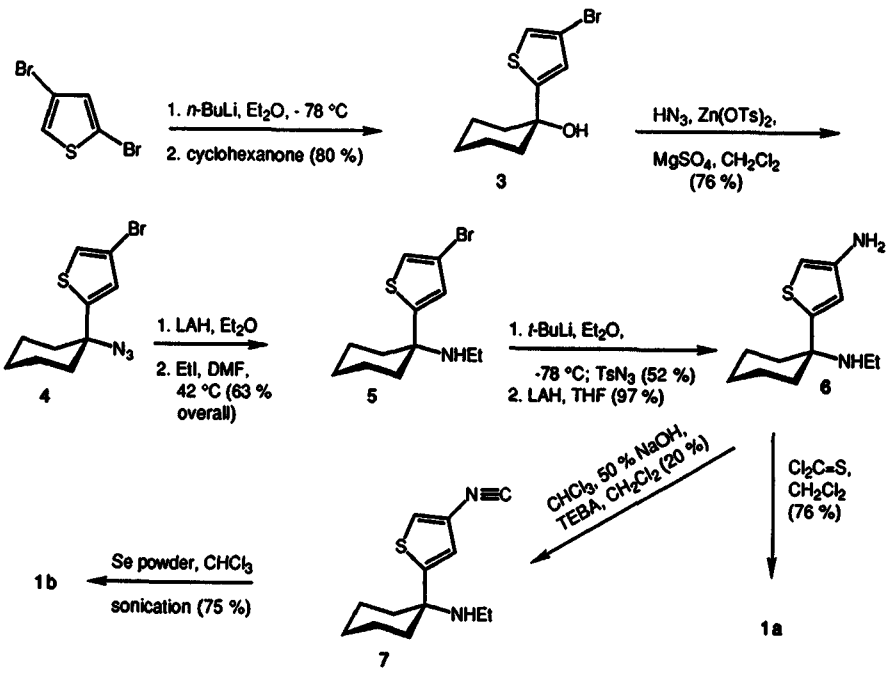
2a, X = S
2b, X = Se

These compounds were synthesized by the addition of 2-lithio-4-bromothiophene[6] to cyclohexanone. The resulting 3° alcohol 3 was converted to the azide 4 by a new method developed in our laboratories which consists of stirring the alcohol with 2 equiv. of HN_3, 0.06 equiv. of zinc tosylate, and 0.72 equiv. of $MgSO_4$ in CH_2Cl_2 at room temperature to give 4 in 76 % yield[7].

Next, the azide was reduced to amine and the amine alkylated to provide predominantly 5. Halogen-metal exchange and trapping of the resulting anion with *p*-toluenesulfonyl azide gave the azidothiophene[8]. This azide was reduced directly to the amine 6 by the action of LAH. Exposure of the diamine to one equivalent of thiophosgene in methylene chloride at -78 °C provided the desired isothiocyanate 1a. To acquire the isoselenocyanate 1b, the diamine 6 was first converted to the isonitrile 7 by a phase transfer variant of Hofmann's carbylamine reaction[9]. This isonitrile was then reacted with powdered selenium in chloroform[10]. Sonication greatly improved the rate and yield for the conversion of isonitrile to isoselenocyanate[1b]. The piperidine analogues 2a and 2b were synthesized in a similar manner starting from 4-bromo-2-[1-(1-piperidino)cyclohexyl]-thiophene obtained by the admixture of 4-bromo-2-lithiothiophene first with magnesium bromide and then with 1-(1-piperidino)cyclohexane-carbonitrile (85%). The remaining steps were identical to those described for the N-ethyl series.

The tritiated form of compound 1a was prepared by reacting 4-bromo-2-lithiothiophene with 4-tosyloxycyclohexanone[6] (82%). The resulting 3° alcohol was transformed to azide (85%), and the tosylate group was eliminated by the action of potassium *t*-butoxide (60%). The azide was reduced to amine (92%), and the amine was N-ethylated (71%). After transformation of bromide to amine in the usual way (48% overall), the double bond was tritiated by use of platinum oxide as catalyst. Lastly, the [^{3}H]-amine was transformed to [^{3}H]-isothiocyanate by reaction with thiophosgene.

All of the above compounds have been tested in the MK-801 binding assay. While complete details of the biological studies will be reported separately[11], compound 1a was found to be the *most efficient agent* discovered to date for labeling the TCP/MK-801 binding sites. Compound 1a, which had an IC_{50} of 300 nM in the MK-801 binding assay, was found to block irreversibly up to 80 % of the MK-801 binding sites without affecting the glutamate or glycine binding sites on the NMDA receptor-complex. In spite of this success, the use of tritiated 1a in the binding experiments revealed a considerable degree of nonspecific binding to membranes. Compounds 1b and 2b were less potent (IC_{50} = 6,300 and 2,500 nM, respectively) than 1a in displacing MK-801 binding and, additionally, failed to exhibit any irreversibility of binding. Rather interestingly, of the analogues reported herein, the isonitrile 7 was found to exhibit the *highest potency* for displacement of MK-801 binding with an IC_{50} of 80 nM. While the isonitrile group could conceivably interact in an irreversible fashion with the PCP/MK-801 binding sites, no irreversibility of binding was found.



In summary, while compound **1a** is not suitable for use in identifying the polypeptides which comprise the NMDA receptor complex, its high alkylating efficiency for the PCP binding sites will nonetheless make it a useful probe in other biological applications[12].

Selected Experimental Procedures:

2-(1-Azidocyclohexyl)-4-bromothiophene (4). To 9.95 g (38 mmol) of **3** in 5 mL of CH_2Cl_2 was added sequentially 54.3 mL (76 mmol) of HN_3 (1.4 M in CH_2Cl_2), 3.3 g (27.4 mmol) of $MgSO_4$, and 0.93 g (2.28 mmol) of zinc tosylate. The mixture was stirred at rt for 4 h, filtered, and concentrated. Chromatography on SiO_2 (hexane, then EtOAc/hexane 1:25) gave 8.3 g (76%) of **4** as a colorless oil: ^1H NMR δ 7.18 (d, 1 H, J = 1.4 Hz), 6.94 (d, 1 H, J = 1.4 Hz), 2.10-2.00 (m, 2 H), 1.90-1.80 (m, 2 H), 1.70-1.50 (m, 6 H); IR (neat) 3112, 2935, 2859, 2100, 1518, 1448, 1332, 1246, 831 cm^{-1}; MS (EI) *m/z* 287/285 (M^+), 245/243, 177/175, 165, 81/79; HRMS calcd for $C_{10}H_{12}{}^{79}BrN_3S$ 284.9935, found 284.9935.

4-Azido-2-[1-(ethylamino)cyclohexyl]thiophene. To a stirred solution of 4.29 g (14.9 mmol) of compound **5** in 175 mL of ether at -78 °C under argon was added 47.1 mL (79.8 mmol) of *t*-butyllithium (1.7 M in pentane) in a fast stream. After 18 min at -78 °C 11.8 g (59.8 mmol) of neat *p*-toluenesulfonyl azide was added dropwise. A thick yellow precipitate appeared. The reaction mixture was stirred at -78 °C for 5 h and then slowly brought to a temperature of -40 °C over 1.5 h. The cold bath was removed and replaced by an ice bath (0 °C). A solution of ethylenediaminetetraacetic acid disodium salt (22.4 g, 40.6 mmol) in 150 mL of water was added. After stirring for 15 min, the ice bath was removed, and the mixture was stirred overnight at room temperature. The reddish-brown ether layer was separated, and the aqueous phase was extracted with ether (3 x 100 mL). The extracts were combined, washed with water and brine, dried ($MgSO_4$), filtered and concentrated under reduced pressure to yield a brown-red oily residue. Chromatography on silica gel using first $CHCl_3$ to remove the excess *p*-toluenesulfonyl azide and then 1:5 EtOAc/hexane gave 1.88 g (52%) of 4-azido-2-[1-(ethylamino)cyclohexyl]thiophene as a slightly colored oil: ^1H NMR δ 6.65 (d, 1 H, J = 1.6 Hz), 6.60 (d, 1 H, J = 1.6 Hz), 2.37 (q, 2 H, J = 7.1 Hz), 1.85 (m, 4 H), 1.60-1.30 (m, 7 H), 1.02 (t, 3 H, J = 7.1 Hz); IR (thin film) 2934, 2853, 2108, 1381, 1253 cm^{-1}; MS (EI) *m/z* 250 (M^+), 207, 179, 126 (100%), 108; HRMS calcd for $C_{12}H_{18}N_4S$, 250.1252, found 250.1253.

2-[1-(Ethylamino)cyclohexyl]-4-isoselenocyanatothiophene (1b). To a solution of 50 mg (0.214 mmol) of **7** in 5 mL of dry chloroform was added under N_2 100 mg (1.26 mmol) of Se powder. The suspension was sonicated for 1 h and then heated with stirring at 55° C for 72 h. After cooling, the grey selenium residue was filtered off. The filtrate was evaporated and chromatographed on SiO_2 with EtOAc/hexane 1:40 to yield 50 mg (75%) of **1b** as a slightly colored waxy solid: ^1H NMR δ 7.14 (d, 1H, J = 1.4 Hz), 6.79 (d, 1 H, J = 1.4 Hz), 2.35 (q, 2 H, J = 7 Hz), 1.90 - 1.70 (m, 4 H), 1.68 - 1.50 (m, 5 H), 1.40 - 1.20 (m, 2 H), 1.04 (t, 3 H, J = 7 Hz); IR (neat) 3700, 2940, 2855, 2102 cm^{-1}; MS (EI) *m/z* 314 (M^+), 285, 271, 234, 191, 135, 122; HRMS calcd for $C_{13}H_{18}N_2S^{80}Se$ 314.0356, found 314.0356.

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References and Notes

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(7) S_N1-reactive alcohols form azides on treatment with HN_3 under acidic (CF_3COOH) conditions: Balderman, D.; Kalir, A. *Synthesis* **1978**, 24. These conditions were too harsh for 1-(2-thienyl)cyclohexanols; on the other hand, the milder procedure described above fails with less activated alcohols like 1-phenylcyclohexanol.

(8) Rapid addition of excess *t*-BuLi was essential to prevent quenching of the thienyl anion by a proton derived from the ethylamino function. The use of *n*-BuLi as base or THF as solvent failed to provide the desired product.

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(12) The structures contained herein are drawn with the thiophene ring axial to represent the conformation which we believe to be adopted at the PCP recognition sites: see, Kozikowski, A. P.; Pang Y. P. *Mol. Pharmacol.* **1990**, *37*, 352.